

Specific Therapeutic Schemes of Omeprazole Affect the Orientation of *Helicobacter pylori*[†]

Marina Azevedo-Vethacke, Désirée Garten, Claudia Groll, and Sören Schreiber*

Ruhr-Universität Bochum, Institut für Physiologie, Im Lottental 36, 44801 Bochum, Germany

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Until now, it has been unclear how proton pump inhibitors (PPIs) support *Helicobacter pylori* therapy. We tested whether the PPI omeprazole acts on the spatial orientation of *H. pylori* in the gastric mucus of infected Mongolian gerbils. Following repetitive PPI administration once daily but not following single doses or administration every 8 h, the bacterial spatial distribution changed, indicating a loss of orientation. Therefore, the therapeutic scheme of PPI administration may affect efficiency of treatment.

The gastric pathogen *Helicobacter pylori* infects about half of all humans (15, 22) and causes ulcers (14) and gastric adenocarcinomas (4, 7). To cure *H. pylori* infection, a combined treatment with antibiotics and a proton pump inhibitor (PPI) for gastric acid suppression is used. Despite frequent administration in conventional triple therapies (12) and the sequential therapies currently being developed (8, 9, 13, 23), the effect of PPIs in therapy for this infection is poorly understood. A finding of major importance was that combined treatment with a PPI causes an increased concentration of the regularly used antibiotic clarithromycin in the gastric mucus (11). Due to the interdependent regulation of acid and mucus secretion, a PPI

may reduce mucus secretion (10). In the case of substances with low gastric clearance rates, decreased mucus secretion after administration of a PPI could enhance their concentrations in the mucus. However, this observation does not explain the mechanism of action of PPIs, since antibiotic schemes without the macrolide clarithromycin (using, e.g., the deeply penetrating fluoroquinolone moxifloxacin [1, 16]) are similarly effective.

Consequently, we tested the new hypothesis that the representative PPI omeprazole may act on the spatial orientation of *H. pylori* within the gastric mucus. We established a chronic *H. pylori* SS1 infection in the Mongolian gerbil, *Meriones unguicu-*

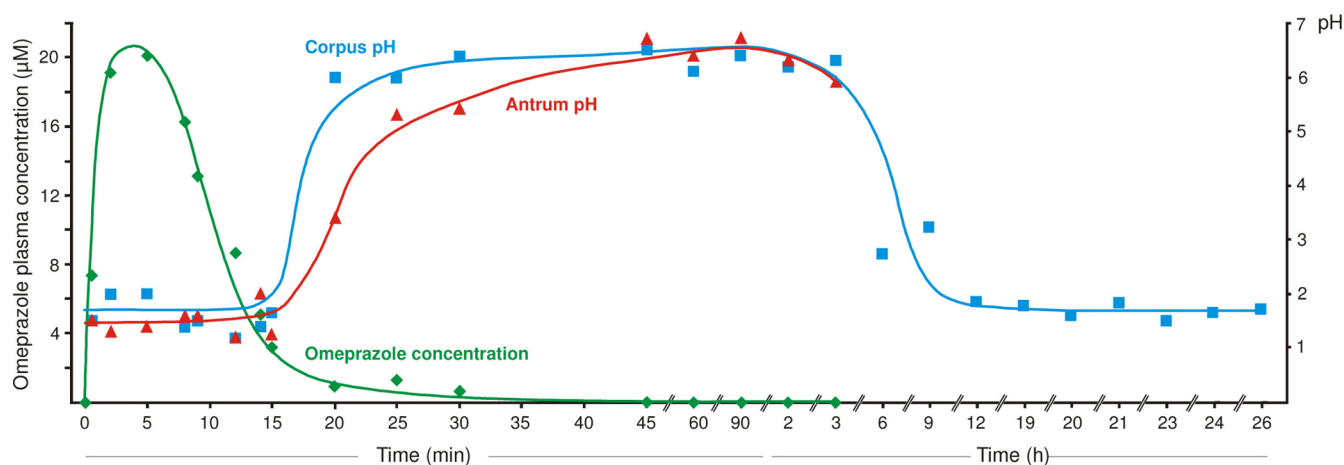


FIG. 1. Course of omeprazole plasma concentration and gastric lumen pH following PPI injection. The omeprazole plasma concentration (green line; measured by high-pressure liquid chromatography) and the changes in the gastric lumen pH (red and blue lines) are shown following an i.p. injection of omeprazole in the Mongolian gerbil. The omeprazole was eliminated within less than 1 hour after administration. The neutralization of the gastric lumen pH, initially detectable in the corpus region, began 15 to 20 min following the i.p. injection, extended to the antrum region, and reached a gastric pH of nearly 7. The luminal pH started to decrease 3 hours after omeprazole administration, and within 12 h, acid secretion recovered.

* Corresponding author. Mailing address: Institut fuer Physiologie, Ruhr-Universität Bochum, Im Lottental 36, 44801 Bochum, Germany. Phone: 49-234-32 29126. Fax: 49-234-32 14191. E-mail: soeren.schreiber@rub.de.

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TABLE 1. Numbers of bacteria observed within different mucus layers after different schemes of PPI administration

Treatment	No. of bacteria/nl of mucus in layer (mean \pm SD)				No. of samples analyzed
	Juxtamucosal		Central	Luminal	
	0–15 μ m	15–25 μ m			
None (control)	4,560 \pm 400	2,280 \pm 430	90 \pm 60	60 \pm 50	206
Single dose of 20 μ M omeprazole through dialysis	5,050 \pm 980	2,010 \pm 310	70 \pm 30	50 \pm 30	86
Two i.p. injections of solvent, 24-h interval	5,740 \pm 1,310	2,260 \pm 400	40 \pm 20	70 \pm 60	142
Two i.p. injections of omeprazole, 8-h interval	4,170 \pm 560	1,800 \pm 440	30 \pm 20	30 \pm 10	147
First dose of omeprazole, second dose of solvent, 24-h interval	4,790 \pm 840	2,030 \pm 520	140 \pm 130	50 \pm 30	101
Two i.p. injections of omeprazole, 24-h interval	3,310 \pm 560 ^a	2,230 \pm 840	300 \pm 130 ^a	120 \pm 30 ^a	143

^a Significant difference by Student's *t* test, *P* \leq 0.05 (for more information, see Table SA2 in the supplemental material).

latus (Hsd:MON) (Harlan and Winkelmann, Indianapolis, IN); administered single and repetitive doses of omeprazole; and studied the effect on the bacterial density and distribution within the gastric mucus layer. Single doses of up to 20 μ M omeprazole were administered by intraperitoneal (i.p.) perfusion, which functioned as dialysis. Repetitive doses of 10 μ M omeprazole were injected i.p. during a short inhalation of anesthesia. Figure 1 shows the omeprazole plasma concentration and the gastric lumen pH after i.p. administration of 20 μ M omeprazole (for details, see the supplemental material).

After the last injection, the bacterial distribution in the mucus was measured in nanoliter samples of mucus from the antrum region to determine the number of colonizing bacteria. By using digital microscopic imaging, it was possible to reconstruct the bacterial distribution with respect to the tissue surface as previously described (19, 21).

The numbers of bacteria per nanoliter of mucus observed within the different mucus layers following different schemes of PPI administration are shown in Table 1. Single doses of up to 20 μ M (35 μ g/ml) omeprazole and two subsequent doses of 10 μ M (0.25 mg i.p.) given at intervals of 8 h did not affect bacterial orientation. Nearly all bacteria were located within

the juxtamucosal mucus layer, with a larger percentage in the first 15 μ m above the tissue surface. The bacterial density and distribution within the different mucus layers in these groups were similar to those of the untreated control animals. Thus, a loss of orientation did not occur.

However, administration of 0.25 mg omeprazole twice with an interval of 24 h resulted in a decreased density of bacteria colonizing the juxtamucosal mucus layer and an increase in the density of bacteria colonizing the central and luminal mucus layers, indicating that the bacteria had spread into these more luminal mucus layers (Table 1).

H. pylori bacteria are normally found to be aligned parallel to one another and the tissue surface in the juxtamucosal layer (Fig. 2A). However, after two subsequent doses of omeprazole once daily, *H. pylori* bacteria were distributed throughout the juxtamucosal, central, and luminal mucus layers in an unarranged manner (Fig. 2B).

Further administration of 0.25 mg omeprazole every 24 h for up to 5 days resulted in a cumulative reduction of the bacterial load to below 5% of the colonization density in the juxtamucosal mucus observed in controls.

Thus, omeprazole affected the orientation of *H. pylori* only

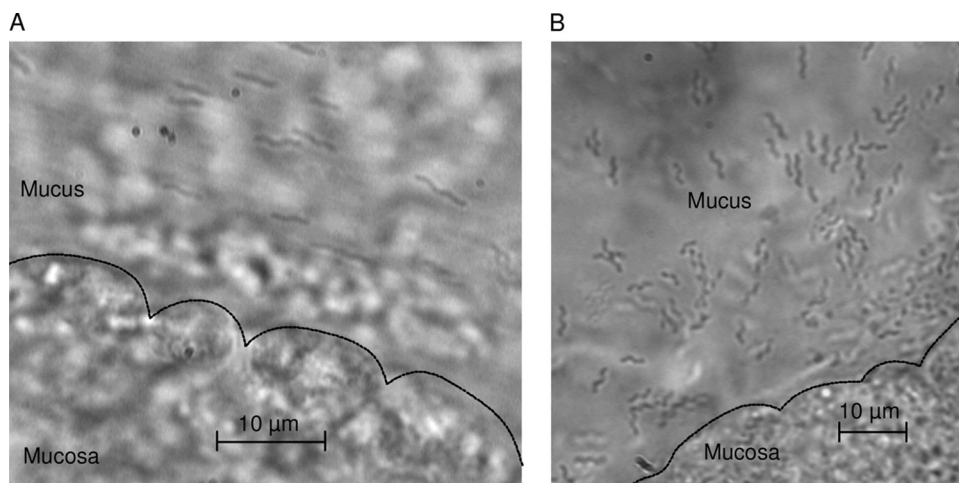


FIG. 2. Spatial alignment of *H. pylori* within the mucus under normal conditions and following omeprazole administration. Shown is the juxtamucosal mucus of the *H. pylori*-infected Mongolian gerbil at a 1,400-rsp, 900-fold magnification. Panel B is a digital addition of three focus planes lying upon another. Epithelial cells of the mucosal surface are visible at the bottom of the micrographs. (A) In untreated animals, the bacteria moved parallel to the cellular surface, within a distance of 0 to 25 μ m. (B) Following 2 days of PPI administration, once daily, the bacteria lost their sorted alignment and spread over the entire mucus layer.

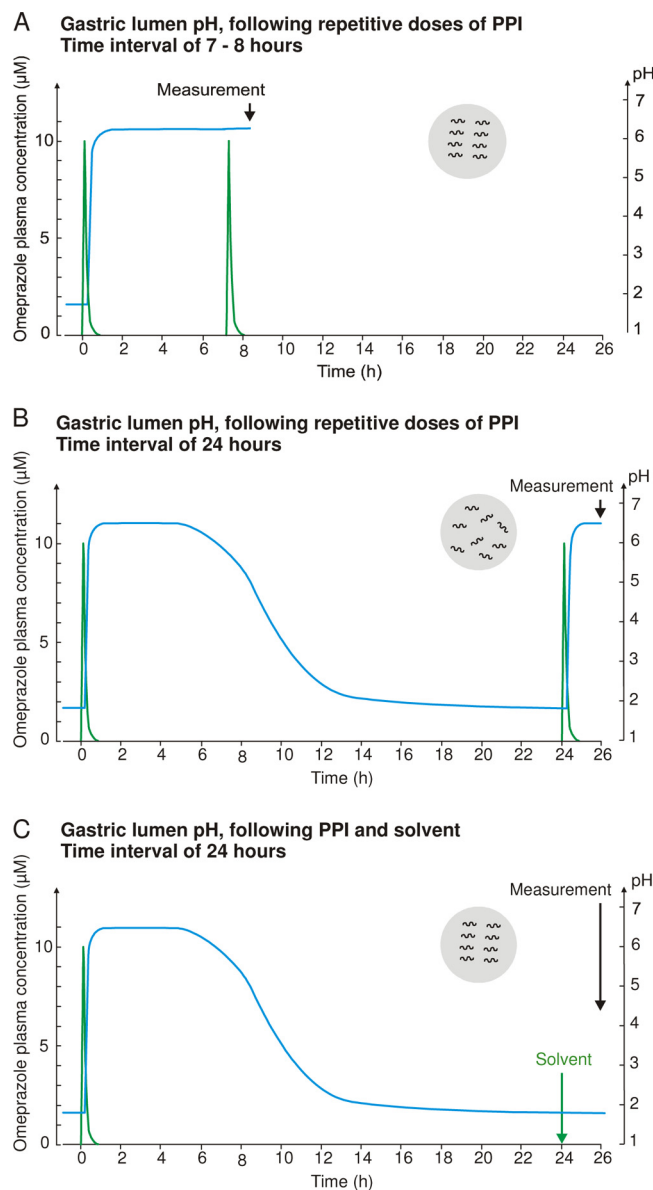


FIG. 3. Omeprazole plasma concentration, gastric lumen pH, and effect on bacterial orientation following different therapeutic schemes. The courses of omeprazole plasma concentration (green line) and gastric lumen pH (blue line) are shown following a peak plasma concentration of 10 μ M omeprazole. (A) The administration of omeprazole at intervals of 8 hours caused a long-lasting neutralization and had no effect on bacterial orientation. (B) Following an interval of 24 h after administration, acid secretion recovered, and the second dose of omeprazole led to a rapid reneutralization of the gastric lumen, inducing a loss of bacterial orientation. (C) When the second dose of PPI was replaced with a solvent, the orientation of *H. pylori* was not affected.

when two or more doses were administered at intervals of 24 h, as the bacterial density and distribution in the juxtamucosal, central, and luminal mucus layers were significantly different in these animals compared to the animals treated with the other schemes (see significance data in Table SA2 in the supplemental material). These observations may result from a modification of the bicarbonate-dependent mucus pH gradient, which

guides *H. pylori* orientation within the gastric mucus layer (19). Due to the continuous mucus flow caused by mucus secretion in the glands and degradation at the luminal surface (20), a precise bacterial orientation is required to prevent *H. pylori* from being vertically swept away into the lumen. The pH gradient in the mucus layer from an acidic lumen pH to a more neutral pH at the epithelial surface is generated by the simultaneous secretion of mucus, bicarbonate, and acid. *H. pylori* senses the local acidity of this pH gradient by using the chemotaxis receptor TlpB (6) and relays the information to the flagellar motor (3). Acid suppression by the PPI may alter the bicarbonate and pH regulation in the gastric antrum, which would in turn disturb the *Helicobacter* orientation. Interestingly, neither a single dose of omeprazole nor two subsequent doses administered at short time intervals contributed to a loss of bacterial orientation. However, when the second (and further) omeprazole dose(s) was given at a regular interval of 24 h, a loss of bacterial orientation was observed. Hence, the long-lasting neutralized gastric lumen pH following one or two doses administered in a shorter interval does not act on *Helicobacter* orientation, whereas the changes in the course of gastric pH values following the second dose given after an interval of 24 h are fatal for *H. pylori*. Figure 3 shows the omeprazole plasma concentration and the gastric luminal pH following a single dose of PPI in comparison to the effect of two subsequent administrations either every 8 or every 24 h.

PPI administration once daily disturbed the vertical orientation of *H. pylori*, most likely through a complex time course of acid suppression, thereby destroying the guiding bicarbonate-dependent mucus pH gradient. *Helicobacter pylori* bacteria which lose their guiding gradient in the gastric mucus spread over the entire mucus layer and into the lumen (19). Depending on the actual pH, the remaining activity of pepsin C in the lumen causes a loss of *H. pylori* motility (17, 18), the bacteria are irretrievably lost, and the bacterial load is reduced. In contrast, two subsequent PPI administrations in shorter time intervals, in which the gastric pH remained neutralized, had no effect on *H. pylori* orientation. Therefore, the time course of the gastric lumen pH that was particularly fatal for bacterial orientation was characterized by a recovery of acid secretion after the last PPI dose and a rapid neutralization following the next dose. These experimental data match the remarkable clinical observations that lower doses (2) or lower frequencies (5) of PPI administration result in improved or unchanged eradication rates.

We conclude that repetitive omeprazole administration every 24 h causes complex changes in gastric acid secretion which impair the bacterial orientation. This finding indicates that the therapeutic scheme of PPI administration may be of importance for an efficient cure of *H. pylori* infection, a finding which requires further clinical examination.

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We declare that no competing interests exist.

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